Label-Free Cancer Cell Separation from Human Whole Blood Using Inertial Microfluidics at Low Shear Stress

Myung Gwon Lee,† Joong Ho Shin,† Chae Yun Bae,† Sungyoung Choi,‡ and Je-Kyun Park*†§

† Department of Bio and Brain Engineering, Korea Advanced Institute of Science and Technology (KAIST), 291 Daehak-ro, Yuseong-gu, Daejeon 305-701, Republic of Korea

‡ Department of Biomedical Engineering, Kyung Hee University, 1732 Deogyeong-daero, Giheung-gu, Yongin-si, Gyeonggi-do 446-701, Republic of Korea

§ KAIST Institute for the NanoCentury, 291 Daehak-ro, Yuseong-gu, Daejeon 305-701, Republic of Korea.

* To whom correspondence should be addressed. E-mail: jekyun@kaist.ac.kr. Phone: +82-42-350-4315. Fax: +82-42-350-4310.
**Measurement Setup and Analysis.** The particle fluid containing beads and cells; and focusing fluid were injected into the CEA microchannel using syringe pumps (KDS200; KD Scientific Inc., Holliston, MA) at specified flow rates with corresponding Re from ~4 to ~16, constantly sustaining the flow ratio between the particles and focusing flow as approximately 1:13–20. The trajectories of the particles and cells were visualized using a fluorescence microscope (TS100; Nikon Co., Japan) equipped with a charge-coupled device (DS–2MBWc; Nikon Co.) with a long exposure time of 8 s or less.

The acquired images with the fluorescence microscope were processed with ImageJ software (http://rsb.info.nih.gov/ij/). The mean lateral position ($Y_{\text{Mean}}$) of the particles was calculated according to the following formulation:

$$Y_{\text{Mean}} = \frac{\sum_{i=1}^{n} y_i I_i}{\sum_{i=1}^{n} I_i}$$  \hspace{1cm} (1)

where $y_i$ refers to pixels (between 0 and 1) measured relative to the lateral position in the contraction region, and $I_i$ refers to the grayscale values of each pixel. The calculated values of each pixel are converted for representing the lateral position of the channel. The separation resolution, $R_s$, between two different sized particles is defined as $R_s = 0.5(\Delta Y/(\sigma_1 + \sigma_2))$, where $\Delta Y$ is the distance between mean lateral position of two particles and $\sigma$ is the standard deviation of lateral position of each kind of particle.
Figure S1. Experimental results of separation efficiency depending on contraction length of 1,200 µm and 300 µm (1/4 contraction length of the proposed design). (a) MCF-7 cell recovery rate is over 90% in the contraction length (CL) of 1,200 µm at every condition of flow rate. (b) Blood cell rejection ratio shows similar trend at every condition of flow rate, but in the CL of 300 µm over 6 mL/h, over 90% blood cell rejection ratio was achieved.
Figure S2. Computational fluid dynamics (CFD) simulation results for (a) straight channel and (b) proposed CEA channel for calculation of shear rate in a microchannel. (a) The condition of straight channel for simulation was performed based on the design demonstrated by Hur et al.\textsuperscript{23} (channel width = 40 µm, height = 93 µm, Re = 21). A maximum x-axial shear rate (Shear U) is calculated to be $4.7 \times 10^4$ /s. And, cells flowing through the straight channel are exposed to the calculated shear rate for 0.119 s in this condition. (b) In the proposed CEA microchannel, a maximum x-axial shear rate is calculated to be $4.9 \times 10^4$ /s in the expansion region and $9.0 \times 10^4$ /s in the contraction region. Cells are exposed to the calculated shear rates for total of 0.078 s as they flow through the CEA device.
Figure S3. (a) Schematic of two step filtration CEA microchannel for CTC isolation from whole blood. Injected blood cells were wasted into the bottom outlet throughout the first and second filtration part. Consequently, the injected cancer cells were collected into the upper outlet throughout the first and second filtration part. (b) Micrograph image of cancer cell (MCF-7 breast cancer cell) isolation from human whole blood (hematocrit level of ~45%) in the CEA microchannel. The MCF-7 cancer cells were stained with green color for visualization. The separation efficiency was evaluated with a throughput (~1.1 × 10^8 cells/min), a MCF-7 cancer cell recovery rate (98.2%), and a blood cell rejection ratio (97.4%).